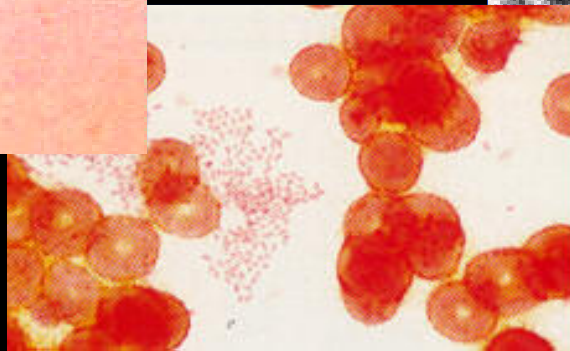
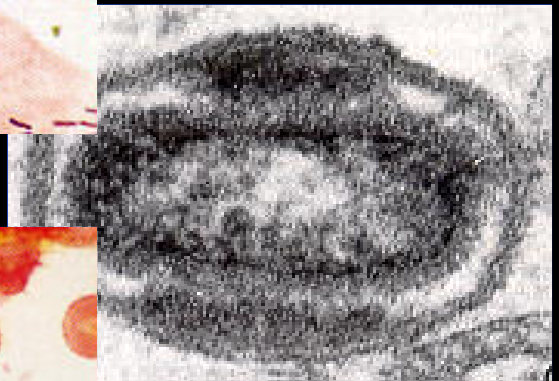
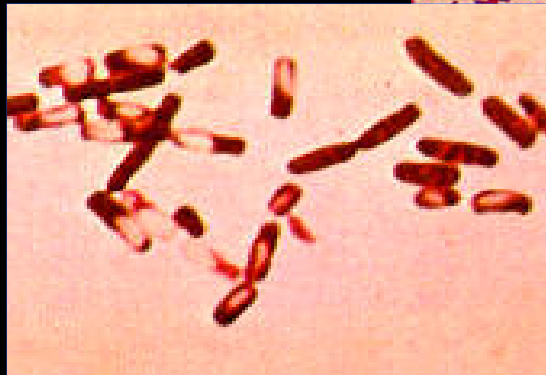




Agents of Bioterrorism





Subject Matter Experts at CDC

- + *Bacillus anthracis* - **Robbin Weyant**
- + *Brucella* spp - **Robbin Weyant**
- + Botulinum toxin - **Susan Maslanka**
- + *Francisella tularensis* - **May Chu**
- + Hemorrhagic fevers - **Tom Ksiazek**
- + Smallpox - **Joe Esposito**
- + *Yersinia pestis* - **May Chu**



Level A Laboratory: Definition

- + BSL-2 Laboratory with a certified Class II biological safety cabinet**
 - BSL-1 microbiology practices plus**
 - Directed by competent scientists**
 - Personnel specifically trained in handling pathogenic agents**
 - Biological safety cabinet, Class II**
 - Access limited by lab director**



Level A Laboratory: Definition, con't

- + BSL-2 Laboratory with a certified Class II biological safety cabinet**
 - Physical containment practices to minimize infectious aerosols
 - “Sharps” precautions
 - PPE (lab coat, gloves, face shield)
 - Biohazard warning signs
 - Biosafety manual defining waste/ S&H/ decontamination/surveillance policy/ CC



Role of the Level A Laboratory





Bioterrorism Agents: Laboratory Risk

<u>Agent</u>	<u>BSL</u>	<u>Laboratory Risk</u>
B. anthracis	2	low
Y. pestis	2	medium
F. tularensis	2/3	high
Brucella spp.	2/3	high
Botulinum toxin	2	medium
Smallpox	4	high
Viral Hemorrhagic fever	4	high



Francisella tularensis

Tularemia



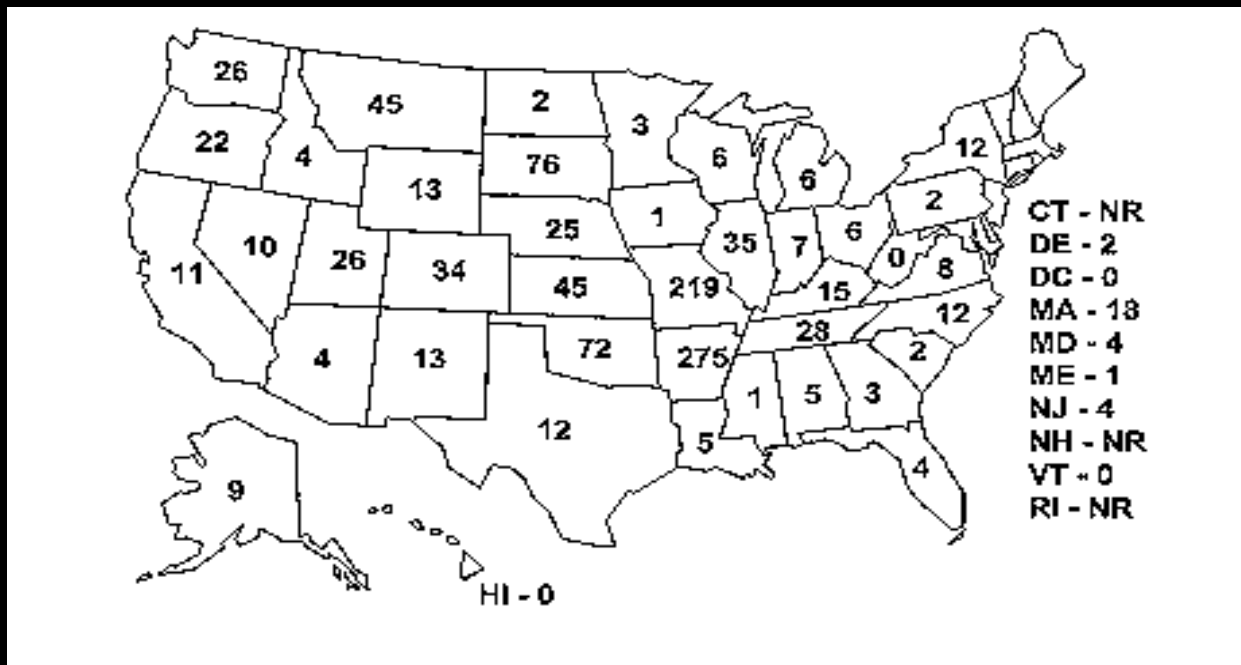
Francisella tularensis

“A Rose by Any Other Name”

- + Plague-like disease in rodents (California)
- + Deer-fly fever (Utah)
- + Glandular tick fever (Idaho and Montana)
- + Market men's disease (Washington, DC)
- + Rabbit fever (Central States)
- + O'Hara's disease (Japan)
- + Water-rat trappers disease (Russia)



Reported Cases of Tularemia - 1990-1998





Level A Procedures ***Francisella tularensis***

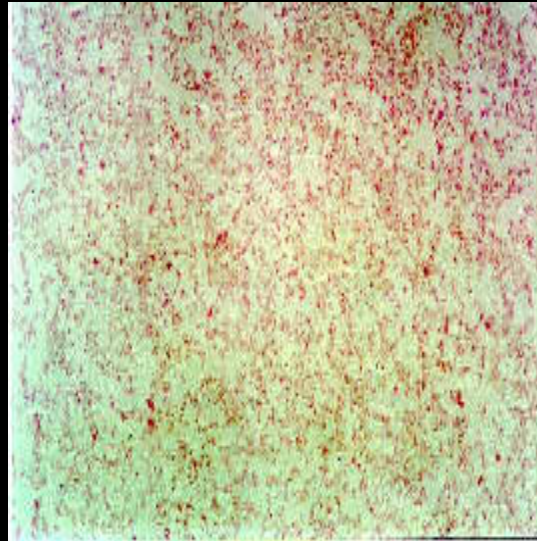
- + *This is a dangerous, highly virulent organism and it should not be manipulated at the bench. Laboratory-acquired infections can occur easily.***
- + Gram stain**
- + Growth characteristics in broth**
- + Growth characteristics in agar**



Francisella tularensis

+ Gram stain

- Poorly staining, tiny Gram-negative coccobacilli**





Francisella tularensis **Growth Characteristics**

- + Fastidious, requires cysteine for robust growth: Cysteine Heart Agar (CHA) is ideal**
 - Enriched chocolate agar : 9% sheep blood + cysteine**
 - Not part of Level A routine procedures**
 - BCYE (for Legionella) also works**



Francisella tularensis **Growth Characteristics**

- + Will grow initially on sheep and chocolate blood agar and Thayer-Martin agar, but poorly or not at all on passage
- + Grows slowly at 35°C, poorly at 28°C



Francisella tularensis

Growth Characteristics

continued

+ 24 hours on SBA, CA, TM, CHA

- gray-white, translucent colonies
- usually too small to be seen individually



Francisella tularensis **Growth Characteristics**

continued

+ 48 hours on SBA, CA, TM, CHA

- SBA - <1 mm, gray-white, opaque, no hemolysis
- TMA, CA - 1-2 mm, gray-white, flat, entire, smooth, shiny
- CHA - 2-4 mm, greenish-white, dense, shiny, opalescent sheen



Gram Negative Coccobacilli

+ Most likely

- Acinetobacter (ox.neg)
- Actinobacillus (sticky)
- H. aphrophilus
- Bordetella, Grp. IV (inert, urea pos)
- Pasturella (nonsticky, Mac pos)

+ Least likely

- DF-3
- Brucella (Urea pos in seconds - minutes)
- Francisella (Urea neg)



Francisella tularensis **Rapid Method Results**

- + Not on the data base of MicroScan or Vitek or API**
- + Should not be worked with in the Level A lab**



Tularemia

- + Contagious --- no
- + Infective dose --- 10-50 organisms
- + Incubation period --- 1-21 days (average=3-5 days)
- + Duration of illness --- ~2 weeks
- + Mortality --- treated: low
untreated: moderate
- + Persistence of organism --- months in moist soil
- + Vaccine efficacy --- good, ~80%



Francisella tularensis **Technical Hints**

If you see:

- + Tiny, gram-negative coccobacilli from blood, lymph node aspirate, or respiratory specimens**
- + Blood isolates that will grow slowly on chocolate agar but poorly or not at all on blood agar in 24 hours**
- + Faint growth in thio; requires cysteine in other broth**



Yersinia pestis

Plague



Plague Epidemiology

- + U.S. averages 13 cases/yr (10 in 1998)
- + 30% of cases are in Native Americans in the Southwest. 15% case fatality rate



Plague Epidemiology

continued

- + Most cases occur in summer and near the patient's residence**
 - bubonic (infected lymph nodes)**
 - septicemic (blood-borne organisms)**
 - pneumonic (transmissible by aerosol; deadliest)**



Yersinia pestis **Specimen Selection**

- + Specimen selection is important**
 - Bubonic - **bubo** - **lymph node aspirate**
 - Septicemic - **blood** - **organisms may be intermittent. Take three specimens 10-30 minutes apart**
 - Pneumonic
 - **Sputum/throat** - **use Wayson and DFA stain**
 - **Bronchial washings** - **Wayson and DFA stain**



Yersinia pestis **specimen inoculation**

**+ Inoculate routine plating media and
make thin smear for DFA**

**Use Wayson only if DFA is
unavailable**



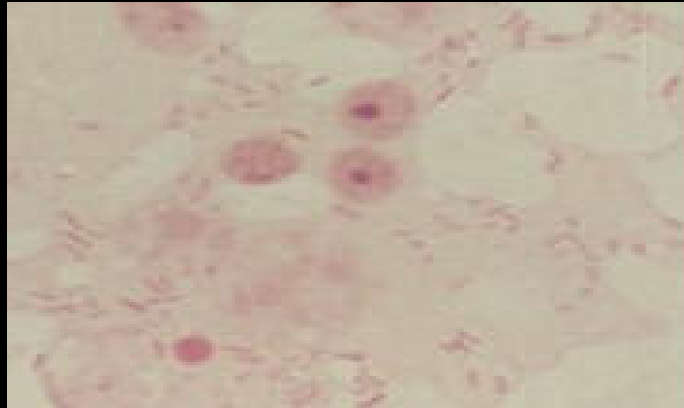
Level A Procedures ***Yersinia pestis***

- + Gram stain**
- + Wayson stain**
- + Growth characteristics on agar**
- + Growth characteristics in broth**



Yersinia pestis **Gram stain**

+ Small, gram-negative bipolar-stained coccobacilli



Must confirm by DFA and mouse inoculation



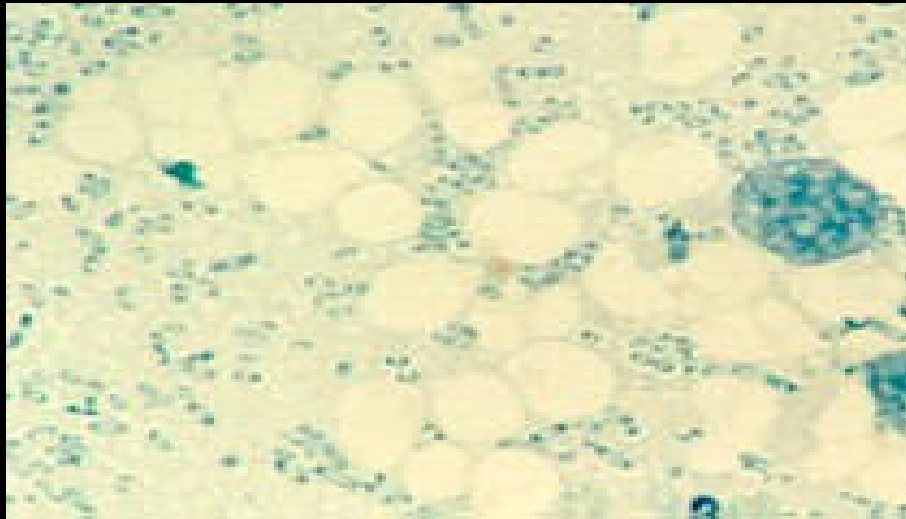
Yersinia pestis **Wayson Stain**

- + Used for rapid assessment**
 - for specimens when DFA is not available
 - when it is a part of the identification process
- + Best with tissue, sputum, blood**
- + Stains of pure culture isolates tend to lose bipolarity**



Yersinia pestis **Wayson Stain**

+ Pink-blue cells with a closed safety pin look



Wayson stain alone is not diagnostic



Yersinia pestis **Growth in Broth**

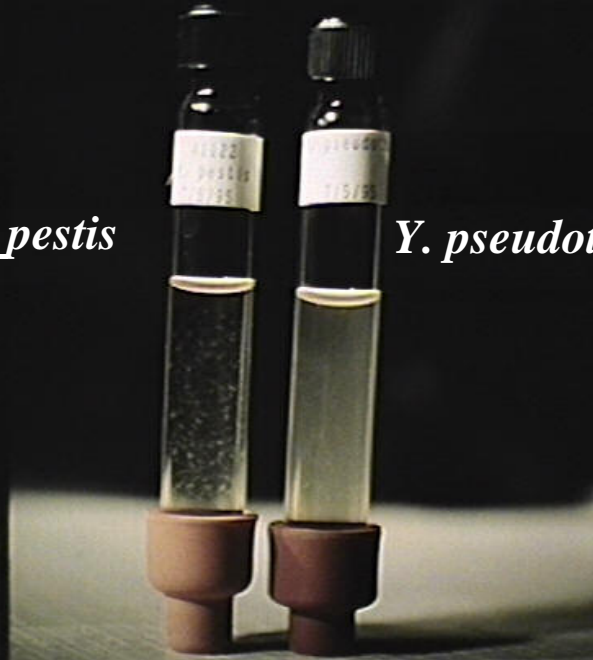
- + Brain Heart Infusion Broth (two tubes)**
 - Incubate at 28°C (best) and 37°C for 24-48h
 - Do not shake tubes
 - Observe suspended flocculent clumps like “stalactites” on side and bottom of tube. Broth remains clear



Yersinia pestis in Broth

Y. pestis

Y. pseudotuberculosis





Yersinia pestis **Growth on Agar**

**+ Sheep blood agar - 28°C (faster)
and 37°C (for DFA tests)**

Looks like other enterics



Yersinia pestis **Rapid Method Results**

- + On the data base of MicroScan, Vitek, and API 20E**
- + True accuracy not yet determined**



Yersinia pestis **Technical Hints**

- + Small gram-negative, poorly staining rods from blood, lymph node aspirate, or respiratory specimens**
- + Safety pin appearance in Gram, Wright, Giemsa, or Wayson stain**
- + More than one patient in a short, specified period with fever, lymphadenopathy**



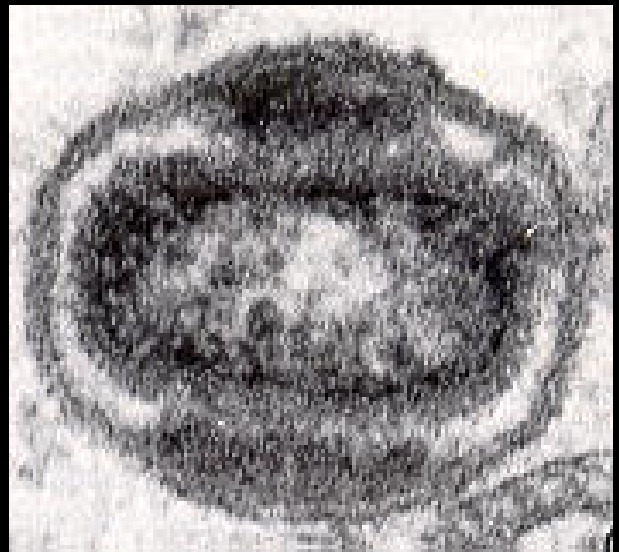
Variola virus

Smallpox



Variola Smallpox virus

- **Large DNA virus**
- **Dumbbell-shaped core**
- **Complex membranes**





Variola Smallpox virus

- + The family Poxviridae consists of eight genera and a few unclassified species
- + Two species are human viruses
 - **Variola virus (genus *Orthopoxvirus*)**
 - **Molluscum contagiosum virus (genus *Molluscipoxvirus*)**
- + ***Orthopoxvirus* includes vaccinia (a lab virus), monkeypox, cowpox, and buffalopox**



Smallpox virus

- + Stored stocks to be retained until 2002 by U.S. and Russia**
 - Undeclared virus could be anywhere**
- + No cases in over 20 years**
- + Controversial decision**
- + Immunity lost in U.S. population**
 - Highly vulnerable to infection**



Level A Procedures Smallpox virus

- + Rule out chickenpox (PCR)!**
- + Specimen of choice is lesion material from pustules.**
 - Collect vesicular fluid from each single lesion**
 - Place droplet fluid as a drop on a clean slide - Do not smear**
 - Store each slide in separate slide holder**
 - Capillary tubes or dry swabs are alternatives**



Level A Procedures Smallpox virus

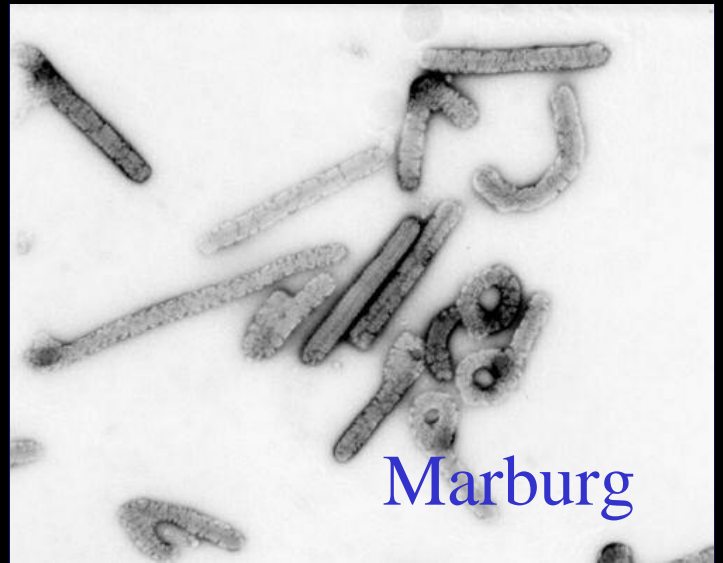
- + Autopsy specimens must be frozen. Formalin fixation is OK for histopathologic study**
- + Contact CDC for approval to ship**
- + Send slide in a non-breakable holder. Do not use a “transport fluid”.**
- + Store at 4°C briefly or at -20°C to -70°C**
- + Decontaminate with 0.5% hypochlorite**



Hemorrhagic Fever Viruses



Ebola



Marburg



Hemorrhagic Fever Viruses

Arenaviruses

- + Argentine HF
- + Bolivian HF
- + Sabia Associated HF

Lassa fever

Lymphocytic
choriomeningitis

Venezuelan HF

Bunyaviruses

- Crimean-Congo HF
- Rift Valley Fever
- Hantavirus Pulmonary Syndrome HF with
0Renal Syndrome



Hemorrhagic Fever Viruses (continued)

Filoviruses

Ebola HF
Marburg HF

Flaviviruses

Tick-borne
Encephalitis
Kyasanur
Forest Disease
Omsk HF



Viral Hemorrhagic Fevers

- + Contagious --- Moderate
- + Infective dose --- 1-10 particles
- + Incubation period --- 4-21 days
- + Duration of illness --- 7-16 days
- + Mortality ---variable
- + Persistence of organism --- unstable
- + Non-endemic in U.S.
- + Vaccine efficacy --- no vaccine



VHF Specimens

+ Diagnosis is clinical, not laboratory



Handling VHF Specimens

- + No specimen accepted without prior consultation - 404-639-1115**
- + Serology - 10-12 ml; 5 ml minimum**
 - serum drawn at admission (acute, convalescent 21 days later; post-mortem heart blood**
 - ship serum cold or on dry ice in a plastic tube**



Handling VHF Specimens- Immunohistochemistry

- + Prefer lung, kidney, spleen tissue
- + Other: lymph node, heart, pancreas, pituitary, brain, liver
- + Paraffin blocks preferred. Formalin-fixed tissue acceptable
- + Ship blocks/tissue at RT - do not freeze. Autopsy/surgical report required.



Handling VHF Specimens- PCR/Virus isolation

- + Ante-mortem - biopsy of lung or bone marrow aspirate or clot
- + Post-mortem - spleen, lung, kidney, liver, nodes, heart, pancreas, pituitary, brain, liver
- + Must be at least 1 cm³
- + Buffy coat, clot, tissue - dry ice



Brucella spp.

Brucellosis



BRUCELLOSIS

- + A zoonotic disease caused by any of 4 *Brucella sp.: abortus, melitensis, suis, and canis*
- + A systemic infection characterized by an undulant fever pattern
- + But relatively rare in the U.S. with approximately 100 cases/yr



BRUCE LLOSIS — by year, United States, 1966-1996



After peaking at more than 300 cases in 1975, the number of brucellosis cases has declined and, for the last 10 years, has remained relatively stable at approximately 100 cases per year.



BRUCELLOSIS: HISTORY

- + 1887 Bruce - Malta fever, *M. melitensis*
- + 1897 Bang - cattle abortion, *B. abortus*
- + 1914 Traum - sow, *B. suis*
- + 1920 Evans, Meyer, Shaw - Brucella
- + 1954 *B. suis*, first weaponized U.S. agent
- + 1968 Carmichael - Beagles, *B. canis*



BRUCELLOSIS: TRANSMISSION

- + Unpasteurized dairy products**
 - The most common mode of transmission
- + Direct skin contact**
 - Occupational hazard for farmers, butchers, veterinarians, and laboratory personnel
- + Aerosols**
 - Highly infectious



BRUCELOSIS

- + Infective dose = 10 -100 organisms**
- + Incubation period = 5 days - > 6 months**
- + Duration of illness = weeks to months**
- + Fever, profuse sweating, malaise, headache and muscle/back pain.**
- + Person to person transmission = no**
- + Mortality = <5%**
- + Persistence of organism = very stable**